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**Response surface model of the effect of pH, sodium
chloride and sodium nitrite on growth of *Yersinia*
enterocolitica at low temperatures**

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Abstract

A fractional factorial design was used to measure the effects and interactions of temperature (5, 12, 19°C), pH (4.5–8.5), sodium chloride (0.5–5%) and sodium nitrite (0–200 µg/ml) on the aerobic growth of *Y. enterocolitica* in brain heart infusion broth. Growth curves were modeled by fitting plate count data to the Gompertz equation. Quadratic models of natural logarithm transformations of the Gompertz *B* and *M* values and the derived values for lag phase durations and generation times were obtained using response surface analysis. Predictions based on the models for *B* and *M* values were comparable to predictions based on the derived values. These models provide a means for rapidly estimating how the bacterium is likely to respond to any combination of the four variables within the specified ranges.

Keywords: *Yersinia enterocolitica*; Low temperatures; Aerobic growth kinetics; Model; Predictive microbiology

1. Introduction

The association of human illness with consumption of food contaminated with *Yersinia enterocolitica* is well documented (Doyle and Cliver, 1990; Lee et al., 1990;

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* Corresponding author.

Kapperud, 1991). *Yersinia* are psychrotrophic, and able to multiply in refrigerated foods (Gill and Reichel, 1989; Doyle and Cliver, 1990; Kapperud, 1991). The microorganism has been isolated from a variety of different food types, including vegetables, dairy products, and pork (Doyle and Cliver, 1990; Lee et al., 1990; Kapperud, 1991), though not all of these isolates contain the virulence associated plasmid. Chilled foods rely on the growth-retarding effect of reduced temperatures to extend the shelf-life. However, since *Y. enterocolitica* is psychrotrophic, control of this foodborne pathogen in refrigerated foods requires a clear understanding of the interaction of temperatures with secondary barriers such as pH, water activity, or atmosphere composition. This knowledge would allow better estimates of the shelf life of products to ensure use before psychrotrophic pathogens such as *Y. enterocolitica* had an opportunity for significant growth.

This need to quantitatively consider multiple barriers can be achieved through recent advances in predictive microbiology. A number of models relating to the growth of *Y. enterocolitica* at sub-optimal temperatures and/or pH in the presence of different acidulants have been described (Adams et al., 1991; Alber and Schaffner, 1992; Little et al., 1992a,b). Using spectrophotometric techniques in conjunction with a large inoculum, Hudson (1993) developed response surface models for the growth of two *Y. enterocolitica* strains as a function of temperature, pH, sodium chloride, and sodium nitrite. We have studied the effects of pH, sodium chloride and sodium nitrite on growth of *Y. enterocolitica* at low temperatures (5–19°C) from low inocula using viable counts. The objective of the current study was to develop response surface models from these data and to compare the models with those of Hudson (1993).

2. Materials and methods

Bacteria. Three plasmid-bearing virulent strains GER (Serotype O:3), PT18-1 (Serotype O:5, O:27), and WA (Serotype O:8) representing three serotypes of *Y. enterocolitica*, were kindly provided by W.E. Hill, Food and Drug Administration. The avirulent plasmidless isogenic derivatives were obtained by isolating large, flat white colonies which emerged from plasmid-bearing cultures grown at 37°C on Congo red brain heart infusion agarose as described by Bhaduri et al. (1991). These isogenic plasmidless strains were used because they grow faster than their corresponding plasmid-bearing virulent strain, thereby insuring appropriately conservative models.

Culture conditions. A mixture of the strains was used. The inocula were prepared by inoculating separate 10-ml test tubes containing 5 ml of Brain Heart Infusion broth (BHI) (Difco Laboratories, Detroit, MI) with individual strains and incubating overnight (18–24 h) at 28°C. Each strain grew to a population density of approximately 10^{10} cfu/ml. Equal volumes of the three cultures were combined. The mixed culture was then diluted using 0.1% peptone water, and used to inoculate the experimental media. The target inoculum level was 2×10^3 cfu/ml.

The culture techniques employed were identical to those described by Buchanan et al. (1989). The culture medium used was BHI broth modified to have the pH and sodium chloride levels described in Table II. The basal BHI medium contained 0.5% NaCl (w/v) and pH of the medium was 7.3. This was modified by adjustment of pH using HCl or NaOH and by addition of appropriate amounts of crystalline NaCl. Triplicate 50-ml portions of medium were dispensed in 250-ml Erlenmeyer flasks and autoclaved for 20 min at 121°C. After cooling, the desired concentration of NaNO₂ was added as a filter-sterilized stock solution. After inoculation with 0.1 ml of the diluted mixed culture, the flasks were incubated on rotary shakers (160 rpm) at the different experimental temperatures.

Variables and experimental design. The following variables were studied in conjunction with a fractional factorial design for four variables: temperature (5, 12, 19°C), pH (4.5–8.5, in 1.0-pH unit increments), NaCl (0.5 to 5%, in 1.5% increments), and NaNO₂ (0–200 µg/ml in 50-µg increments).

Bacteriology. At appropriate intervals, samples were removed, and diluted as needed in 0.1% peptone water and immediately surface plated with a Spiral Plater (Model D, Spiral Systems, Bethesda, MD) on duplicate BHI agar (Difco) plates. After 24 h incubation at 37°C, colonies were counted with a Laser Bacteria Colony Counter (Spiral Systems Instruments, Inc., Bethesda, MD).

Certain combinations of variables prevented growth. 'No growth' was defined as a count at the end of the incubation period of 21 days at or below the starting count.

Curve fitting. Growth curves were generated from the experimental data using the Gompertz equation (Gibson et al., 1988) (Table 1) in conjunction with ABACUS, a non-linear curve-fitting regression program developed by W.C. Damert (Eastern

Table 1
Gompertz equation and derived kinetic values

Gompertz equation	
$L(t) = A + C \exp(-\exp(-B(t - M)))$	
$L(t)$	log ₁₀ count bacteria at time t (h) [log ₁₀ (cfu/ml)]
A	asymptomatic log count of bacteria as t decreases indefinitely (initial count of bacteria, [log ₁₀ (cfu/ml)])
C	asymptomatic amount of growth that occurs as t increases (number of log cycles of growth, [log ₁₀ (cfu/ml)])
B	relative growth rate at time M (h ⁻¹)
M	the time at which the absolute growth rate is maximal (h)
Derived growth kinetic values	
EGR	exponential growth rate = BC/e [log ₁₀ (cfu/ml)/h]
GT	generation time = $\log_{10} 2e/(BC)$ (h)
LPD	lag phase duration = $M - (1/B)$ (h)
MPD	maximum population density = $A + C$ [log ₁₀ (cfu/ml)]

Table 2

Comparison of observed growth kinetics values with those predicted by the kinetics parameters-based and Gompertz parameters-based models (Table 3)

Independent variables				Observed kinetics values								Kinetics parameters based models ^b		Gompertz parameters based models ^c			
Temp.	pH	NaCl	NO ₂	<i>A</i>	<i>C</i>	<i>B</i>	<i>M</i>	EGR	GT	LPD	MPD	LPD ^a	GT ^a	<i>B</i>	<i>M</i>	LPD ^a	GT ^a
5	4.5	0.5	0	1.90	8.60	0.0044	832.38	0.014	22.4	599.0	10.5	489.9	17.0	0.0058	636.52	464.0	18.3
5	4.5	2.0	0	2.44	NG	0.0000	–	–	–	–	–	784.7	24.9	0.0052	832.87	642.3	20.2
5	5.5	0.5	0	2.60	7.80	0.0079	205.83	0.023	13.3	78.6	10.4	75.1	7.9	0.0128	168.83	90.7	8.3
5	5.5	0.5	50	1.50	8.60	0.0112	351.25	0.035	8.5	262.0	10.1	108.0	9.6	0.0105	223.65	128.5	10.1
5	5.5	2.0	50	2.00	8.10	0.0070	393.10	0.021	15.3	241.5	10.1	201.6	13.8	0.0088	325.77	211.5	12.1
5	6.5	0.5	0	2.80	7.40	0.0202	74.23	0.055	5.5	24.7	10.2	29.1	5.9	0.0179	88.36	32.4	5.9
5	6.5	0.5	150	2.70	7.60	0.0203	72.10	0.057	5.3	22.7	10.3	38.3	7.3	0.0149	114.42	47.4	7.1
5	6.5	2.0	0	2.90	7.30	0.0196	94.89	0.052	5.9	42.1	10.2	61.1	8.0	0.0140	138.25	66.9	7.6
5	6.5	3.5	0	2.80	6.40	0.0207	146.74	0.048	6.4	97.1	9.2	106.3	10.6	0.0149	178.58	111.6	7.1
5	6.5	5.0	0	2.70	2.00	0.0283	151.63	0.021	14.5	116.1	4.7	153.5	13.6	0.0216	190.43	144.2	4.9
5	7.5	0.5	0	2.80	7.50	0.0196	79.93	0.054	5.6	28.4	10.3	28.6	7.1	0.0158	91.24	27.8	6.7
5	7.5	0.5	200	2.80	7.40	0.0200	73.21	0.054	5.6	23.3	10.2	21.2	6.8	0.0180	77.72	22.0	5.9
5	7.5	2.0	100	3.00	7.00	0.0074	289.56	0.019	15.9	153.9	10.0	93.9	11.0	0.0100	196.58	96.9	10.6
5	8.5	0.5	0	3.00	7.50	0.0070	235.99	0.019	15.9	89.2	10.5	70.9	13.5	0.0088	185.90	71.9	12.1
12	4.5	0.5	0	2.50	8.20	0.0199	122.79	0.060	5.0	72.6	10.7	79.4	5.7	0.0152	147.59	82.0	6.9
12	4.5	0.5	50	2.60	9.14	0.0179	115.13	0.061	4.9	58.4	11.7	118.6	7.1	0.0120	202.82	119.7	8.8
12	4.5	3.5	0	2.50	NG	0.0000	–	–	–	–	–	159.7	11.6	0.0136	230.10	156.8	7.8
12	6.5	0.5	0	2.50	7.60	0.0382	35.32	0.107	2.8	9.2	10.1	9.9	2.4	0.0388	33.35	7.6	2.7
12	6.5	0.5	200	2.80	7.60	0.0407	34.72	0.114	2.6	10.2	10.4	8.5	2.5	0.0379	32.91	6.6	2.8
12	6.5	5.0	0	2.80	6.80	0.0227	116.41	0.056	5.4	71.2	9.6	47.9	5.2	0.0338	83.26	53.7	3.1
12	6.5	3.5	200	2.80	7.00	0.0174	76.69	0.045	6.7	18.9	9.8	33.9	4.9	0.0231	84.62	41.4	4.6
12	7.5	5.0	200	2.70	6.40	0.0172	165.78	0.040	7.4	107.6	9.1	86.2	6.5	0.0224	139.33	94.7	4.7
19	5.5	0.5	0	2.30	8.50	0.0801	22.37	0.249	1.2	9.9	10.7	5.9	1.1	0.0963	16.28	5.9	1.1
19	5.5	0.5	50	2.00	8.80	0.0719	22.05	0.232	1.3	8.1	10.8	8.2	1.3	0.0806	20.69	8.3	1.3
19	5.5	0.5	100	1.90	8.80	0.0707	21.88	0.229	1.3	7.7	10.7	9.1	1.4	0.0737	22.92	9.4	1.4
19	5.5	0.5	150	2.10	8.60	0.0714	22.17	0.226	1.3	8.2	10.7	8.1	1.4	0.0737	22.13	8.6	1.4
19	5.5	0.5	200	2.10	8.60	0.0696	23.19	0.221	1.4	8.8	10.7	5.9	1.3	0.0806	18.61	6.2	1.3
19	5.5	2.0	100	3.00	7.40	0.0612	28.71	0.166	1.8	12.2	10.4	16.3	1.9	0.0487	37.49	17.0	2.2
19	6.5	3.5	150	3.20	7.40	0.0405	44.12	0.110	2.8	19.1	10.6	20.2	2.0	0.0494	43.42	23.2	2.1
19	7.5	0.5	0	3.10	7.60	0.0947	17.31	0.263	1.1	6.7	10.7	9.9	1.4	0.0809	23.32	11.0	1.3
19	7.5	5.0	0	3.10	7.60	0.0910	17.73	0.255	1.2	6.7	10.7	66.1	2.6	0.0410	88.18	63.8	2.6
19	7.5	5.0	200	3.10	7.00	0.1166	47.80	0.299	1.0	39.1	10.2	51.1	2.6	0.0437	78.84	55.9	2.4

^a Calculated using grand mean for $C = 7.73$. ^b Calculated using Eqs. 3 and 4, Table 3. ^c Calculated using Eqs. 1 and 2, Table 3.

Regional Research Center, U.S. Department of Agriculture, Philadelphia, PA). This program employs a Gauss–Newton iterative procedure. Typically, the Gompertz A value was fixed at the experimental value obtained for the 0-h sample. The Gompertz parameters (B , C , B) were subsequently used to calculate lag phase duration (LPD), exponential growth rate (EGR), generation time (GT), and maximum population density (MPD) and ‘time to a 1000-fold increase’ (T_{1000}) as described previously (Buchanan and Phillips, 1990; Buchanan and Klawitter, 1992).

Model development. Polynomial models for the effect of temperature, pH, sodium chloride content and sodium nitrite concentration were calculated for natural logarithm (Ln) transformations of the Gompertz B and M parameters, and Ln transformations of the derived LPD and GT values calculated for individual growth curves. All models were generated using the SAS General Linear Model procedure (SAS, 1989).

3. Results and discussion

Prior to response surface model development, two sets of analyses were performed to assess which of the Gompertz parameters would be modeled. First, the effect of inoculum size on the growth kinetics of *Y. enterocolitica* was examined. Inoculum levels of 10^3 to 10^5 cfu/ml were employed in conjunction with BHI containing 5% NaCl and 0 $\mu\text{g/ml}$ NaNO_2 at pH 7.5 with cultures being incubated at 19°C. Little, if any effect on the growth kinetics (LPD and GT) of *Y. enterocolitica* could be attributed to differences in inoculum levels (data not shown). Similar results have been reported with other Gram-negative and Gram-positive food-borne pathogens including *Salmonella*, *Listeria monocytogenes*, *Aeromonas hydrophila*, *Shigella flexneri*, and *Escherichia coli* O157:H7 (Buchanan and Phillips, 1990; Buchanan et al., 1993a,b; Gibson et al., 1988; Palumbo et al., 1991; Whiting, 1993; Zaika et al., 1992). This allowed exclusion of the Gompertz A term from consideration.

The second supplemental analysis was an evaluation of the effect of the independent variables on the MPD. As noted for several other pathogens (Buchanan and Phillips, 1990; Buchanan et al., 1993a; Palumbo et al., 1991, 1992; Whiting, 1993; Zaika et al., 1992), the MPD attained by the microorganism was generally independent of the cultural variables (Table 2). If *Y. enterocolitica* grew, it typically achieved an MPD of between 10^9 and 10^{11} cfu/ml. Only when a combination of two or more variables approached values that prevented growth was there any depression of MPD below 10^9 cfu/ml. Therefore, C , A , and MPD could be estimated by the grand mean of the experimental values (7.73, 2.55, and 10.28, respectively), and were not modeled.

The influence of the four independent variables on the growth kinetics of the microorganism was evaluated by generating 96 growth curves representing 32 combinations of the variables (Table 2). Both EGR and LPD were influenced by temperature, pH, and NaCl. T_{1000} values increased with decreasing temperature.

Table 3

Second-order response surface models on temperature (T , 5–19°C), pH (P , 4.5–8.5), NaCl (S , 0.5–5.0%) and NaNO₂ (N , 0–200 µg/ml) for aerobic growth of *Y. enterocolitica*

Gompertz parameters models

– B value [1]

$$\text{Ln}(B) = -15.2641 + 0.1569T + 3.1817P + 0.0310S - 0.0102N - 0.0136TP - 0.1047TS + 0.0000276TN \\ - 0.0479PS + 0.0010PN - 0.0002SN + 0.0028T^2 - 0.2297P^2 + 0.0681S^2 + 0.0000178N^2$$

$$R^2 = 0.882$$

– M value [2]

$$\text{Ln}(M) = 22.7381 - 0.3846T - 4.9289P - 0.0058S + 0.0135N + 0.0348TP + 0.0047TS - 0.00017N \\ + 0.0596PS - 0.0012PN + 0.0002SN + 0.0010T^2 + 0.3398P^2 - 0.0426S^2 - 0.00003N^2$$

$$R^2 = 0.928$$

Derived kinetics parameters models

– Lag phase duration [3]

$$\text{Ln}(LPD) = 28.8310 - 0.5589T - 6.8269P + 0.0262S + 0.0161N + 0.0528TP - 0.0027TS - 0.00017N \\ + 0.0900PS - 0.0012PN + 0.0002SN + 0.0037T^2 + 0.4642P^2 - 0.0415S^2 - 0.0000427N^2$$

$$R^2 = 0.901$$

– Generation time [4]

$$\text{Ln}(GT) = 12.9571 - 0.2132T - 3.1630P + 0.3918S + 0.0093N + 0.0122TP - 0.0011TS - 0.00004617N \\ - 0.0255PS - 0.0008PN + 0.0002SN + 0.0002T^2 + 0.2352P^2 - 0.0064S^2 - 0.0000165N^2$$

$$R^2 = 0.904$$

increasing sodium chloride levels, and acid or alkaline pH values. While growth was observed over the temperature (5–19°C), pH (4.5–8.5), and NaCl (0.5–5%) ranges tested, two combinations of suboptimal conditions prevented growth: 5°C–pH 4.5–2% NaCl/0 µg/ml NaNO₂ and 12°C–pH 4.5–3.5% NaCl–0 µg/ml NaNO₂. Overall, the effect of sodium nitrite on the growth of *Y. enterocolitica* was relatively small. This is in contrast with the results of Hudson (1993) who reported that sodium nitrite had a marked effect on the growth kinetics of both a type strain and a food isolate of *Y. enterocolitica*.

The data were used to develop two sets of quadratic response surface models; one based on the natural logarithm (Ln) transformation of the Gompertz B and M parameters and the other on the Ln transformation of the LPD and GT derived from Gompertz equation (Table 3). Ln transformation has been used extensively in model development as means of stabilizing the variances of the experimental values (Alber and Schaffner, 1992; Buchanan and Phillips, 1990; Buchanan et al., 1993a,b; Palumbo et al., 1992). The R^2 values for both sets of models indicated adequate fits despite the relatively small number of variable combinations that were analyzed. Comparisons of observed and predicted T_{1000} values indicated reasonable agreement for both sets of models (Figs. 1 and 2), indicating that both sets of regression models provide reasonable agreement with the observed effects and interactions of the four variables on the growth parameters of *Y. enterocolitica* within the limits of the experimental data. Only one variable combination, 19°C – pH 7.5 – 5.0% NaCl – 0 µg/ml NaNO₂, was substantially outside the 50% limits due to both models predicting a much longer LPD than observed. It is likely that this can be corrected in future models by increasing the number of variable combinations tested in this region.

Table 4

F-values for independent variables and their cross products for the quadratic models based on Ln transformations

	Ln(<i>B</i>)	Ln(<i>M</i>)	Ln(LPD)	Ln(GT)
Temperature	6.1 **	44.2 *	45.1 *	12.4 *
pH	46.9 *	136.3 *	126.3 *	51.1 *
NaCl	0.0	0.0	0.0	2.3
NaNO ₂	4.6 **	9.7 **	6.7 **	4.3 **
Temperature * pH	3.5	27.8 *	31.0 *	3.1
Temperature * NaCl	5.6 **	1.4	0.2	0.1
Temperature * NaNO ₂	0.1	0.5	0.3	0.3
pH * NaCl	1.0	1.9	2.1	0.3
pH * NaNO ₂	2.2	3.8	1.9	1.8
NaCl * NaNO ₂	0.3	0.9	0.4	0.6
Temperature * Temperature	1.8	0.3	1.8	0.0
pH * pH	41.6 *	110.3 *	99.5 *	48.1 *
NaCl * NaCl	6.8 **	3.3	1.5	0.1
NaNO ₂ * NaNO ₂	2.2	6.3 **	7.4 **	2.1

F-values are based on type II sum of squares (SAS, 1989)

* $P < 0.001$

** $0.05 > P > 0.001$.

Comparison of the magnitudes of the *F*-values (based on type II sum of squares) (SAS, 1989) associated with the models for *B* and *M* and those for LPD and GT were used as a means of evaluating the relative importance of the variables and their cross products (Table 4). The majority of the microorganism's response could be attributed to two of the primary variables, pH and temperature. Relatively little effect was associated with the interaction terms, suggesting that the primary variables were largely independent. Adams et al. (1991) reported that effects of temperature and pH were independent for *Y. enterocolitica*. It should be noted that models based on Ln transformations exclude the no-growth data, which in this case tends to diminish the relative importance of NaCl as a primary factor controlling growth.

Relatively few data are available on the growth of *Y. enterocolitica* in food products at low temperatures with which the effectiveness of predictions by the models could be compared. Comparison of T_{1000} values estimated from three studies with various foods against predictions using the kinetics-parameters-based model indicate that the model provides a reasonable 'first estimate' of the behavior of the pathogen in foods (Table 5). In order to show that these models are valid for growth of *Y. enterocolitica* in foods, additional studies of the growth of this organism in food are required.

Kinetics parameters-based and Gompertz parameters-based models were both generated to evaluate in part, the suggestion of Garthright (1991) that the former should be the better approach. Experience has indicated that models based on the Gompertz *B* and *M* parameters can result in negative values when LPDs are subsequently calculated. Comparison of GT, LPD and T_{1000} values (Table 2 and

Table 5

Comparison of reported times to achieve a 1000-fold increase (T_{1000}) in *Y. enterocolitica* populations in various foods with times predicted by models in Table 3^c

Substrate	Temperature (C)	pH ^a	NaCl (%) ^a	NaNO ₂ (μg/ml) ^a	T_{1000} (days)		Strain	Source
					Exp. data ^b	Model		
Simulated milk	7	6.8	0.5	0	3-4	2.3	Various serotype 0:3 and 0:9	Rowe, 1988
Ground beef	4	5.8	0.5	0	11-12	5.3		Kleinlein and Untermann, 1990
	10	5.8	0.5	0	3.5	1.9		
	15	5.8	0.5	0	1.5	0.9		
Cooked beef	7	5.8	0.5	0	4-5	3.1	Various	Hanna et al., 1977
Raw beef	7	5.8	0.5	0	3-4	3.1		
Cooked pork	7	5.8	0.5	0	4	3.1		
Raw pork	7	5.8	0.5	0	3-4	3.1		

^a Values assumed.

^b Estimated graphically from growth curves.

^c Predictions calculated using the kinetics parameters-based models, assuming a *C*-value of 7.28 and an *A*-value of 3.00, and using the equation: $T_{1000} = \text{LPD} + 7.826 * \text{GT}$.

Hanna et al. (1977)

Kleinlein and Untermann (1990)

Rowe (1988)

Fig. 3) indicated a high degree of correlation between the two approaches. While the use of the derived kinetics parameters does tend to simplify model generation, the current data set indicates that the two approaches appear equivalent in terms of validated final models.

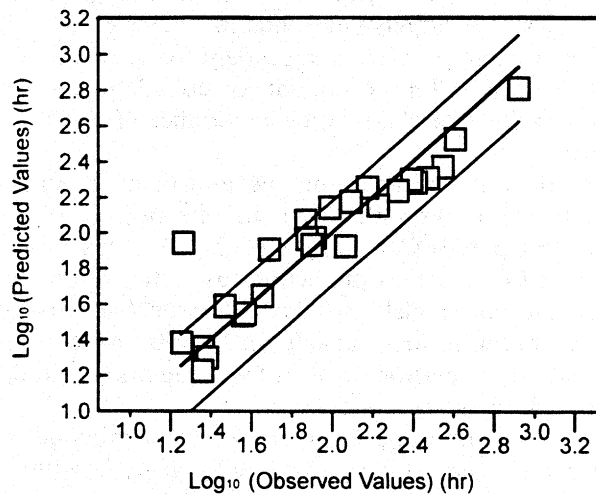


Fig. 1. Comparison of observed T_{1000} values with those predicted using Gompertz parameters-based models. Center line is line of identity, and two exterior lines represent $\pm 50\%$ of observed value. Calculated using the equation: $T_{1000} = (\text{Ln}(-\text{Ln}(3/7.73)))/-B + M$.

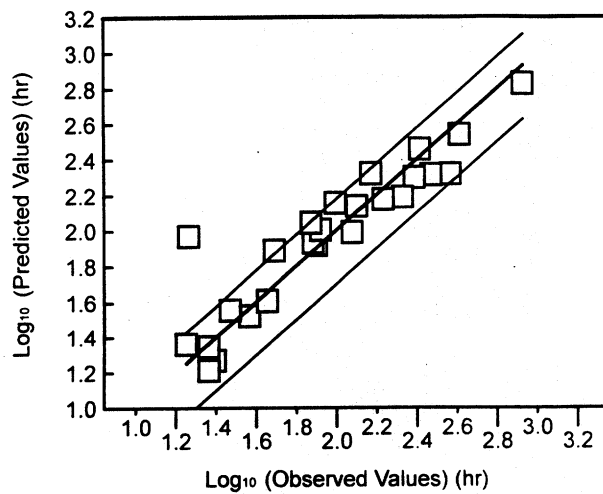


Fig. 2. Comparison of observed T_{1000} values with those predicted by kinetics parameters-based models. Center line is line of identity, and two exterior lines represent $\pm 50\%$ of observed value. Calculated using the equation: $T_{1000} = \text{LPD} + 1.0549 / (0.81828 / 7.73 * \text{GT})$.

The kinetics parameters-based model from the current study was compared with the cubic model of Hudson (1993) that was developed using the type culture, ATCC 9610. While the model of Hudson (1993) encompasses a wider temperature range (4–39°C), its ranges for pH (5.5–7.7), sodium chloride (0–3%), and sodium nitrite (0–125 ppm) were more restricted than the current model. Accordingly,

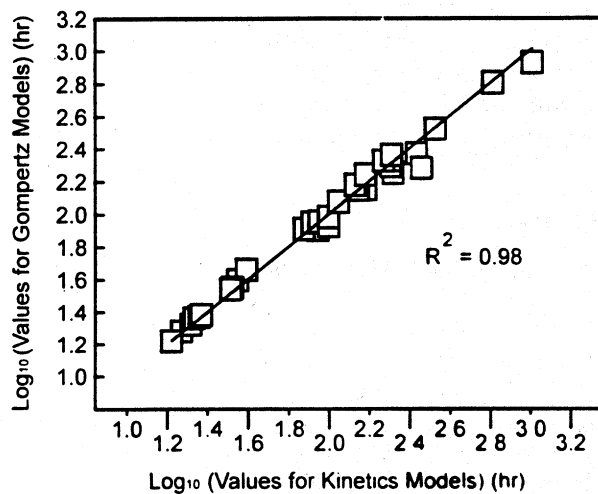


Fig. 3. Comparison of T_{1000} values predicted by kinetics parameters-based and Gompertz parameters-based models.

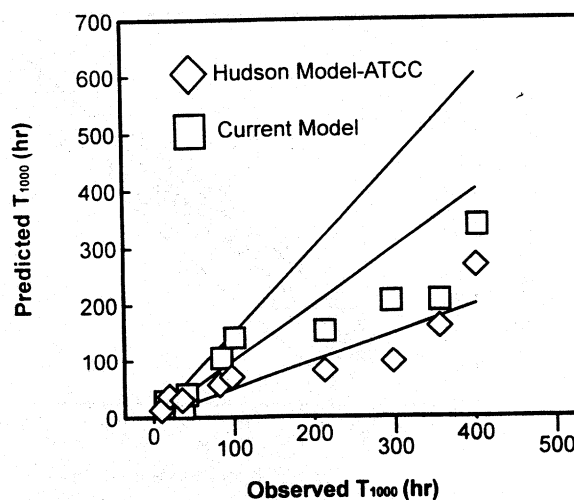


Fig. 4. Comparison of observed versus predicted T_{1000} values for current kinetics parameters-based models and that of Hudson (1993). Center line is line of identity and outer lines represent $\pm 50\%$ of observed value.

there were relatively few experimental data points against which the performance of the two models could be compared (Fig. 4). In general, the models were in close agreement with variable combinations that yielded short T_{1000} values, but the model of Hudson (1993) appeared to consistently underestimate the growth of the microorganisms, particularly under conditions where growth was slow. This could reflect either differences in the strains or cultural conditions employed, or could reflect inherent differences resulting from the two means of assessing growth; viable counts versus absorbance.

The models reported here enable prediction of the effects of combinations of four variables on growth kinetics of *Y. enterocolitica* in a culture medium, and are comparable with models developed in our laboratory for other pathogens. Further work is in progress to extend the model to temperatures higher than 19°C.

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